

*Citation:*

Wolff, L.K., On the formation of antibodies after injection of sensitized antigens. II, in:  
KNAW, Proceedings, 17 I, 1914, Amsterdam, 1914, pp. 318-329

and Delft are absolutely certain, still I believe that the probability is not small, that the difference between the assumed and the real coefficients of expansion of the prototype at Breteuil and the metre N<sup>o</sup>. 27, is for the greater part the cause of the value of  $\Delta L$ . It remains absolutely uncertain, what the real coefficients of expansion of the metres are and also whether the coefficient of N<sup>o</sup>. 27, determined after FIZEAU's method, merits greater or less confidence than that of the prototype deduced, as I believe, from direct measurements at different temperatures. But whatever it may be, it is of great importance, and it is in my opinion the chief result which may be deduced from my discussion, that when a direct comparison of the metre N<sup>o</sup>. 27 and the international metre shall be made, according to the right given to our government, it will not be confined to a comparison at a mean temperature, but that if possible, the absolute coefficient of expansion of our metre, and certainly the difference in expansion of N<sup>o</sup>. 27 and the prototype will also be determined.

*Lenk (Switzerland).*

**Physiology.** — “*On the formation of antibodies after injection of sensitized antigens.*” II. By Dr. L. K. WOLFF. (Communicated by Prof. C. EYKMAN.)

I. As a continuation to my series of experiments given in the first communication, I have examined the immunisation power of a mixture of erythrocytes and specific serum with a surplus of amboceptor.

It is generally stated in literature that this power is very slight or that it does not exist at all; in my two series of experiments I have also found very little or no formation of amboceptor. I shall communicate one of the series.

Horsecorpuscles — specific rabbitserum  $\frac{1}{200}$  strong.

Binding power of 1 c.cm. 5% blood  $\pm 7$  doses.

Mixture of 40 c.cm. serum and 20 c.cm. undiluted blood i. e. 20 doses amboceptor, so a great surplus.

Rabbit 149, 73 and 76 each get 20 c.cm. of the mixture intraperitoneal.

„ 179, 70 and 71 „ „ 6 $\frac{1}{2}$  „ undiluted blood only „

Titre	after 1 day	after 7 days	after 12 days
149	$\frac{1}{10}$ weak	$< \frac{1}{10}$	$< \frac{1}{10}$
73	$\frac{1}{50}$	$\frac{1}{50}$ weak	$\frac{1}{50}$ weak
76	$\frac{1}{10}$	$\frac{1}{10}$ weak	$\frac{1}{20}$
179	—	$\frac{1}{20}$	$\frac{1}{20}$
70	—	$\frac{1}{100}$	$\frac{1}{100}$
71	—	$\frac{1}{100}$	$\frac{1}{100}$ weak.

So with the rabbit 149 and 73 we do not find a trace of active immunisation, only of passive; rabbit 76 after 12 days shows a small (active) increase of titre. The controlling rabbits however have distinctly formed amboceptor.

The second experiment with cattle corpuscles had a perfectly analogous course. With these experiments we cannot inject intravenously; the animals which are intravenously injected with such a great quantity of serum and corpuscles die of anaphylaxis.

II. I have now put to myself the question what happens with the sensitized corpuscles after the injection into the rabbit or cavia.

Therefore I have for the time being confined myself to the subcutaneous resp. subconjunctive injection; the intravenous one is very difficult to follow, the progress of the peritoneal one is mostly known; besides the subcutaneous is the only one that is to be considered with regard to man. I expected that in keeping with what happens in the peritoneum, viz. a solution of the sensitized red corpuscles in a short time, the corpuscles would also dissolve in the subcutaneous tissue. I have taken the conjunctiva as the spot where to inject: there the phenomena are to be controlled better than anywhere else, and one can easily cut out little pieces for microscopic examination.

Well then: if we inject foreign corpuscles under the conjunctiva they are generally gone after one, and certainly so after two days.

As they have no movement of their own, we must assume them to be led away along the lymphpaths — a leading away by phagocytes in such a short time is not to be assumed. It is however different if sensitized corpuscles are injected; these remain on the spot; they do not dissolve in any quantity worth mentioning, and if one microscopies the place after a longer or shorter space of time (after cutting out, fixing, embedding, and colouring) one will find an important number of leucocytes between the corpuscles.

After 6 to 8 days only the corpuscles have generally disappeared; sometimes however they are still to be seen after 10 to 12 days.

During the first few days one mostly finds polynuclear small leucocytes, later more great mononuclear ones.

Now the question is how to explain this conduct. For this we must examine three things.

1<sup>st</sup>. How is it that the sensitized corpuscles which are injected subconjunctively do not dissolve, while those injected intraperitoneally do.

2<sup>nd</sup>. Why do the sensitized corpuscles remain in the same place, whereas the normal ones are carried away.

3<sup>d</sup>. What happens finally to the sensitized cells; what do the leucocytes do.

Let us first answer the first question.

Here we must ask at once if there is complement in the subcutaneous lymph.

As far as I know H. SCHNEIDER's<sup>1)</sup> researches about this subject are the best; he found that the tissue lymph which is obtained by bringing a piece of cottonwool under the skin, and afterwards pressing it out, contains very little complement indeed. One always finds a little more complement than would really be the case if we had pure tissue lymph; a slight mixing with serum can of course hardly be avoided. It goes without saying that in this way we cannot be certain to get a liquid, agreeing with the tissue lymph; the piece of cottonwool naturally works irritating; an inflammation arises. But the injection of the corpuscles also causes an inflammation, and as such these two processes are equal.

I have also made some complement titrations to the guinea pig and rabbit, of subcutaneous fluids obtained in this way.

For the solution of my haemolytic system I needed:

I. Fresh guinea pig serum	$\frac{1}{100}$ c.cm.
Subcutaneous fluid	$\frac{1}{20}$ c.cm.
II. Fresh guinea pig serum	$\frac{1}{50}$ c.cm.
Subcutaneous fluid	$\frac{6}{50}$ c.cm.
III. Fresh rabbit serum	$\frac{1}{4}$ c.cm.
Subcutaneous fluid	0,6 c.cm. no haemolysis!
Stowing fluid	0,6 c.cm. trace of „

So we can affirm SCHNEIDER's experiments and assume very little or no complement to exist in the subcutaneous cellular tissue; and we need not be astonished about the sensitized corpuscles not dissolving, when being injected subcutaneously.

Now we must answer the second question. The sensitized cells remaining in the same place was supposed to be due to the agglutination which always accompanies the sensitizing. I did not succeed in obtaining an immune serum prepared in the usual way, which did not at the same time agglutinate. As I did not know any method to separate amboceptor and agglutinin when I started my experiments, I took another way to prove that the remaining of the bloodcells was owing to their being agglutinated and not to the sensitizing. I therefore agglutinated the bloodcells in a different way, and now found that clinically and histologically the same was to be seen after injecting these corpuscles as after injecting sensitized (and

<sup>1)</sup> Arch. f. Hygiene 70. p. 40 seq.

at the same time agglutinated) cells. In the first place I used a colloidal solution of  $\text{SiO}_2$  for it.

All the red bloodcells I used (rabbit, guinea pig, horse, cattle, dog) were agglutinated by it, be it in various concentration. Only the  $\text{SiO}_2$  had no effect; it caused neither swelling, nor leucocytosis. It had been prepared by saponifying Siliciummethylether (KAHLBAUM) with greatly diluted hydrochloric acid. Colloidal  $\text{SiO}_2$ , prepared in a different way had the same effect. Now one might object against this experiment that the  $\text{SiO}_2$  not only agglutinates the bloodcells, but that it also sensitizes them; for together with guinea pig serum in a great quantity, it can dissolve some kinds of blood. Therefore I took refuge to the vegetable agglutinins which are found in the bean, pea, lentil, and in the seeds of *Datura Stramonium*. In all these cases the result was the same: the bloodcells always remained there; the conjunctiva also showed the wellknown bluish-red change of colour after some days, and histologically the image was always the same. It goes without saying that with all those experiments the sterility was taken into consideration as much as possible.<sup>1)</sup>

In order to make quite sure, however, that only sensitized and agglutinated corpuscles did *not* show the phenomenon, I examined some thirty rabbits out of my collection on haemolysin and agglutinin against sheep-erythrocytes, and I really found some sera which did contain haemolysin, but only little agglutinin. I repeated the experiments with these sera; but the results were not very distinct: there sometimes was a difference, but it was not big enough to draw a certain conclusion from it.

This is because all the sera employed were rather weak (amboceptor  $\frac{1}{50}$ — $\frac{1}{100}$ ) and so a rather big quantity of serum was necessary ( $\pm 3$  cm.) to sensitize the cells. Normal rabbitserum generally containing some agglutinin, we did not succeed in this way in obtaining a suspension of sheep-erythrocytes which are sensitized but little or not agglutinated. Yet I can communicate one experiment which came out rather well:

Serum rabbit	73	titre amboceptor	$\frac{1}{50}$	very little	agglutinin.
„	„	147	„	nearly $\frac{1}{100}$	much

$\frac{1}{2}$  ccm. sheep-erythrocytes is digested with  $\pm 3$  ccm. serum 73, just as  $\frac{1}{2}$  ccm. with  $\pm 3$  ccm. serum 147. The suspensions are centrifuged and the corpuscles are taken up in 1 ccm. salt solution. Erythrocytes 73 are injected on the right, erythrocytes 147 on the

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<sup>1)</sup> I did not use ricine because the poisonous qualities of this substance would have injured the image.

left under the conjunctiva of rabbit 172. The serum of this rabbit contains neither amboceptor nor agglutinin in a noticeable quantity against sheep-erythrocytes.

After one day there is a distinct difference. There is very little swelling and redness (+) on the left, but very strong swelling and bluish-red change of colour (+++) on the right. The next day the difference is a little less, but still it is distinct.

Consequently it was desirable to obtain a serum which sensitized strongly (at least  $1/1000$ ), but which agglutinated little or not at all. As there was no question of a chemical separation — all the literature tells us that all suchlike attempts lead to no result whatever — such serum had to be obtained in a different way. In the literature about the heterogenetical antibodies is mentioned that serum of a rabbit which had been in some way prepared in order to get heterogenetical amboceptors against sheep-erythrocytes, would then contain no more agglutinins than are found in normal rabbitserum.

My experiments in this direction have however not yet led to the desired result. One rabbit which was injected with cattle-erythrocytes had a serum with titre  $1/200$  against cattle-corpuscles, and a titre  $1/1000$  against sheep-corpuscles. However it very clearly contained agglutinins against the latter. The same thing appeared with two rabbits which had been injected with horse-kidney extract. The titre against sheep-blood was  $1/200$  of both of them. Both distinctly contained agglutinins, if only little. The sheep-corpuscles treated with this serum remained for some days in the same place, after having been injected under the conjunctiva.

So in this way I could not prove *with certainty* that the agglutinin is the cause of the prepared corpuscles remaining under the conjunctiva. <sup>1)</sup>

III. I will now mention some experiments which have been made in connection herewith, but which do not directly bear upon the subject mentioned in the title. I have asked myself whether the same difference as is mentioned above, is also found when non-prepared bloodcells are injected subconjunctively partly with prepared, partly with non-prepared animals, and whether here too the agglutinin was of any importance as to the remaining of the erythrocytes. And this has indeed appeared to be the case.

Rabbits with serum containing amboceptor (and agglutinin) still show a strong swelling under the conjunctiva after one or two days after having been injected with the erythrocytes in question

<sup>1)</sup> Note added during to the correction: Now I had more success with this experiment. The heterogenetical serum which I now used was  $1/2000$  strong.

(in my experiments they were horsecorpuscles), whereas the controlling animals showed hardly any swelling after one, and no swelling at all after two days. In accordance with this the tissue fluid (obtained in the above mentioned way with cottonwool) obtains amboceptor as well as agglutinin, if they are in the serum.

Rabbit 160 immunized against cavia-erythrocytes.

*Serum* agglutination  $\frac{1}{50}$  amboceptor  $\frac{1}{10}$  weak (++)  
*fluid* „  $\frac{1}{20}$  „  $\frac{1}{10}$  „ (+).

Rabbit 192 immunized against horse-erythrocytes.

*Serum* agglutination  $\frac{1}{10}$  amboceptor  $\frac{1}{20}$   
*fluid* „  $\frac{1}{10}$  weak „  $\frac{1}{20}$  nearly

Rabbit 147 immunized against cattle-erythrocytes.

*Serum* agglutination  $\frac{1}{5}$  amboceptor  $\frac{1}{50}$   
*fluid* „  $\frac{1}{5}$  „  $\frac{1}{20}$ .

I have now investigated if it really is the agglutinin which determines the difference.

*Rabbit* 116 agglutination strong, amboceptor  $\frac{1}{100}$ .

*Rabbit* 148 „ , very weak, „  $\frac{1}{50}$ .

Both rabbits are subconjunctively injected with  $\frac{1}{2}$  c.cm. washed sheep-erythrocytes.

After one day there is a very strong bluishred swelling with 116, with 148 hardly any swelling; after 2 days still a strong swelling with 116, with 148 nearly all the blood has disappeared.

A stronger proof is given by the rabbits that were injected with horsekidney extract<sup>1)</sup>. Although the titre against sheepcorpuscles was not high here (with both  $\frac{2}{200}$ ) a great difference was stated with the controlling animal (titre also  $\frac{1}{200}$ ).

After one day hardly any blood was to be seen with the first, contrary to the controlling-animal. I think these experiments are of some importance. For in the latest great report about the agglutination known to me, that by PALTAUF<sup>2)</sup>, the author says on p. 515: Ob Agglutination auch im Organismus stattfindet erscheint recht zweifelhaft.

At least I believe I have proved the haemagglutination to take place in the subcutaneous tissue. I only want to insert here that

<sup>1)</sup> These are the same animals as were mentioned above: their serum did contain agglutinin, but much less than the animals immunized in the ordinary way. That here we got no agglutinin effect, and that we did when mixing the serum with the bloodcells in vitro, may be explained by the fact that the agglutinin can pierce with so much more difficulty into the tissue fissures and reach the bloodcells than when a great quantity of serum in vitro is directly added.

<sup>2)</sup> KOLLE und WASSERMANN, 11e Auflage, II, p. 483—654.

the phenomena mentioned above belong to the department of local anaphylaxis (Phenomenon of ARTHUS). As far as I know they have not been studied as to the immunisation with bloodcells; they have with serum or bacteria. This really is only a question of name however: the essence of local anaphylaxis is still as unknown to us as that of general anaphylaxis.

In any case we can see by the bloodcells that the disintegration of albumen is a very slow one; I do not wish to deny however, that part of the flood of leucocytes is owing to this disintegration. What has been stated somewhere else viz., a primary necrosis of the tissue and after that an infiltration of leucocytes<sup>1)</sup>, I have never observed; I could sometimes also state a toxical influence of the injection out of an oedema of the cornea: but this happened very rarely. Then one should not directly compare the phenomena of subcutaneous injection with those of intracorneal injection (WESSELY, von SZILY); in the latter case the current of fluid is much slower, so that great differences can occur by this. It would however lead us too far if we entered into this more closely.

We must now still treat of the third question: what happens to the sensitized (agglutinated) cells, and what do the leucocytes do in this process? I must first of all mention that I could not find any difference between histological images when injecting sensitized or only agglutinated bloodcells. This, however, is in keeping with other experiments. For, there being a great difference in vitro between the phagocytosis of sensitized (opsonized) and nonsensitized cells, — the former are phagocytated, the latter are not, when brought together with suitable leucocytes — one does not find back this difference in vivo when injecting the cells into the abdomen, previously injected with broth. ACHARD and FOIX<sup>2)</sup> some time ago tried to find the causes of this difference, but in vain. I did not succeed either<sup>3)</sup>. We need not be astonished however, when finding the same conduct in the subcutaneous tissue as in the prepared abdomen.

Are the erythrocytes now phagocytated? Notwithstanding my observing a great many preparations, I did not succeed in getting any certainty whatever about this in my histological sections; to form

1) H. FUCHS und MELLER, Z. f. Ophthalmologie. Bd. 87, p. 280.

2) ACHARD and FOIX Arch. de Médecine expérimentale et d'anatomie Pathologique, January 1914.

3) Prof DE VRIES advised me to add to the mixture (foreign bloodcells, fresh serum (without opsonins) and leucocytes) scrapings of the peritoneum endothelium; with this I had no success either.



an opinion about it is, however, very difficult; leucocytes are always among a great number of red cells and the sections are always thicker than one red or white cell. Anyhow, it seems very probable to me that this must happen. For:

1. the red cells disappear after 6—8 days.
2. in vitro they are easily phagocytated.
3. The subcutaneous cellfluid and the leucocyte extract do not contain an unspecific haemolysin (SCHNEIDER: l. c.; this concerns polynucleous (mikrophages) as well as mononucleous cells (macrophages).

I have tried after one or two days to cut out the swelling (after injecting the sensitized (agglutinated) cells), and then to spread them out on a coverglass: these preparations too gave bad images; principally by the stickiness of the substance: I did not see a distinct phagocytosis.

I have here always spoken about sensitized cells without wishing to form an opinion about the open question of identity between amboceptors and opsonins and tropins. (NEUFELD<sup>1)</sup> SATSCHENSKO<sup>2)</sup>).

The following experiment will show that there can be amboceptor as well as tropins in the subcutaneous cellular tissue. A piece of cottonwool was entered under the skin of the abdomen of a prepared rabbit (against sheep-erythrocytes) and the fluid was examined after some hours: in vitro it strongly stimulated the phagocytosis of sheep-erythrocytes by rabbit-leucocytes.

As a summary we can draw the following conclusions:

1. When using red corpuscles loaded with amboceptor as antigen one should remove all surplus of serum.
2. Sensitized and agglutinated red corpuscles, when injected subcutaneously, remain in the same place for a long time; non-treated cells are soon led away.
3. This will most probably be the consequence of the agglutination, not of the sensitizing. The same happens to non-specific agglutination — also when it concerns the animal's own cells.
4. With prepared animals possessing agglutinin, the cells injected also remain in the place where they have been injected. So agglutination in vitro also takes place; this is not the case with animals which only possess amboceptors (opsonins) and no agglutinins.
5. The subcutaneous lymph contains very little or no complement, it does contain amboceptor, agglutinin, opsonin (tropin).

The above will show my experiments not yet to be complete. They require to be completed as to the question to what

<sup>1)</sup> Arbeiten aus den Kaisrl. Gesundh. Bd. 25, 27 en 28.

<sup>2)</sup> Arch. Sc. biol. St. Petersburg. XV, blz. 145 1910.

extent the immunizing power of red corpuscles loaded with antibodies is related to that of normal cells as to the tropin- and the agglutinin-content of the serum. We may suppose, also in consequence of the above mentioned experiments, that the content of antibodies of serum and subcutaneous lymph goes parallel and so we shall not investigate this point separately.

IV. After the immunisation with sensitized erythrocytes the one with mixtures of serum and anti-serum comes next. I have not stated the amboceptortitre (to be stated by means of complement fixing) but the precipitincontent. Where the results do not differ much from the experiments with sensitized erythrocytes, I think I can suffice with only stating the precipitin.

IA Rabbits, injected intravenously with horseserum, 0,5 c.c.m. per kg. (made inactive).

Rabbit	weight	titre after 3 days	after 5 days	after 7 days	after 12 ds.	after 14 ds.
103	2600	—	—	—	$\frac{1}{100}$	$\frac{1}{1000}$
104	2850	—	—	—	$\frac{1}{1000}$ weak	$\frac{1}{1000}$
105	2850	—	—	—	$\frac{1}{1000}$ "	$\frac{1}{1000}$
106	1650	—	—	—	$\frac{1}{100}$	$\frac{1}{1000}$ weak
107	2100	—	—	—	$\frac{1}{1000}$ weak	$\frac{1}{1000}$

IB. Rabbits injected, intravenously with 0,5 c.c.m. horseserum (inactive) + 1 c.c.m. precip. serum ( $\frac{1}{1000}$ ), after this mixture had stood for 1 hour.

Rabbit	weight	titre aft. 3 days	after 5 days	after 7 days	aft. 12 days	aft. 14 days
108	2150	—	—	$\frac{1}{10}$	$\frac{1}{100}$	$\frac{1}{100}$
109	2150	—	—	$\frac{1}{10}$	$\frac{1}{1000}$	$\frac{1}{1000}$
111	1850	—	—	—	still some } $\frac{1}{100}$	$\frac{1}{1000}$
111	1850	—	—	—	horse serum } $\frac{1}{1000}$	$\frac{1}{1000}$
112	2150	—	—	$\frac{1}{10}$ weak	$\frac{1}{1000}$ weak	$\frac{1}{1000}$

So here we do not see a distinct difference between the A and B group.

II A. Rabbits, injected intraperitoneally 0,4 ccm. human serum per kg.

Rabbit	Weight	after 5 days	aft. 7 ds.	aft. 10 ds.	aft. 12 ds.	aft. 14 ds.
67	2150	—	—	$\frac{1}{10}$	$\frac{1}{1000}$	$\frac{1}{10000}$
78	2450	—	—	$\frac{1}{10}$	$\frac{1}{100}$	$\frac{1}{1000}$ weak
70	1900	—	—	$\frac{1}{10}$	$\frac{1}{1000}$	$\frac{1}{10000}$
60	2320	—	$\frac{1}{10}$ ?	$\frac{1}{100}$	$\frac{1}{1000}$	$\frac{1}{10000}$
76	1820	—	$\frac{1}{10}$	$\frac{1}{100}$	$\frac{1}{1000}$	$\frac{1}{10000}$

II B. Rabbits injected similarly 0,4 ccm per kg. + 3,6 ccm. antiserum ( $\frac{1}{1000}$  largely). The mixture had stood for 4 hours, a thick precipitate has been formed.

Rabbit	Weight	aft. 5 ds.	aft. 7 ds.	aft. 10 ds.	aft. 12 ds.	aft. 14 ds.
	2100	—	—	$\frac{1}{100}$	$\frac{1}{1000}$	$\frac{1}{1000}$
114	3200	no more human serum.	—	—	—	$\frac{1}{10}$
113	2000		—	—	—	$\frac{1}{10}$
112	2550		—	—	$\frac{1}{1000}$	$\frac{1}{100}$ weak
71	1600		—	—	—	$\frac{1}{1000}$
						$\frac{1}{1000}$

After 17 days the titre already went back.

Here we can see that, whereas of the series B three rabbits distinctly lag behind, two of them reach as high a titre as the A rabbits. Knowing (UHLENHUTH) that accidental failures in the preparation of precipitinholding sera are not to be avoided, I should not wish to draw any other conclusion from this than that a good formation of precipitins is also possible with mixtures of serum and antiserum.

I have also taken the following series of experiments.

III. Rabbit 140 1 c.cm. horseserum intraperitoneal.

„ 116	„	„	„ + 1 ccm antiserum
			( $\frac{1}{1000}$ )
„ 142	„	„	„ + 3
„ 99	„	„	„ + 5
„ 121	„	„	„ + 7
„ 8	„	„	„ + 9
„ 42	„	„	„ + 11
„ 48	„	„	„ + 13

Rabbits	after 8 days
140	—
116	$\frac{1}{100}$ weak.
142	$\frac{1}{1000}$ „
99	$\frac{1}{1000}$
121	$\frac{1}{1000}$
8	$\frac{1}{1000}$
42	$\frac{1}{10000}$
48	—

So here too we find a rather important formation of antiserum with rabbits, which, with the serum, had also got antiserum.

IV. Rabbit 155 50 c.cm. antiserum  $\frac{1}{1000}$  + 2 c.cm. horseserum intraperit.

„ 156	30	„	„	+ 2	„	„	„
„ 157	10	„	„	+ 2	„	„	„
„ 158	0	„	„	+ 2	„	„	„
„ 159	15	„	„	+ 0	„	„	„

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Rabbit	after 1½ hour	after 1 day	after 3 days	after 5 days
	contains horse serum	contains horse serum	contains horse serum	contains horse serum
155	+	+++	++	++
156	+	++	++	++
157	?	—	+?	+?
158	++	+++	++	++
159	—	$\frac{1}{10}$	—	—

  

	after 7 days.	aft. 10 ds.	aft. 12 ds.	aft. 14 ds.	aft. 17 ds.
	contains horse serum	contains horse serum			
155	+	$\frac{1}{10}$	$\frac{1}{100}$	$\frac{1}{1000}$ W.	$\frac{1}{100}$
156	++	$\frac{1}{10}$	$\frac{1}{100}$ weak	$\frac{1}{100}$ W.	$\frac{1}{100}$
157	—	$\frac{1}{10}$	$\frac{1}{100}$	?	$\frac{1}{10}$ W.
158	++	$\frac{1}{10}$	$\frac{1}{100}$	$\frac{1}{100}$	$\frac{1}{100}$
159	—	—	—	—	—

Here too we also see some irregularity: (rabbit 157 immunizes somewhat less than the other, but even a mixture of 25 times more antiserum than serum still has immunizing effect.

I did not try if surplus of serum can do any harm when immunizing, for one then gets too great quantities so that it is hard to inject them: 50 ccm. serum is rather much for a rabbit.

These experiments seem to be somewhat contrary to a communication of DORR (report about Anaphylaxis, KOLLE UND WASSERM. IIe Aufl.), that the precipitate obtained by mixing serum and antiserum, has no immunizing effect. But this is only a seeming contradiction. For, according to investigations e.g. by WELSH and CHAPMANN<sup>1)</sup> this precipitate only contains traces of parts of the serum and it is almost exclusively formed out of the antiserum.

Thus I have found that of a serum of a rabbit which was immunized against human serum (titre  $\frac{1}{1000}$ ) 75 ccm. was necessary to form together with 1 ccm. human serum (together till 150 ccm.) a precipitate, so that in the above mentioned liquid no more human serum could be indicated with my antiserum ( $\frac{1}{1000}$ ). 1 ccm. being a very small dosis to immunize a rabbit, it is clear that not much can be expected in general from an injection of the precipitate<sup>2)</sup>.

I have now also examined the local effect of serum and antiserum.

<sup>1)</sup> Zeitsch. f. Immunitätsf. 9, p. 517.

<sup>2)</sup> I here give up the question whether there is any human serum at all to be found in the precipitate, or whether it could be again removed by washing.

With this the antiserum and serum were always both inactive, so that we have nothing to do with any possible anaphylatoxin.

If one again injects the mixture in which a precipitate has been formed subconjunctively, one will find a rather strong swelling the next few days, which at a morphological examination again seems to contain polynucleous cells. The controlling animals which had only been injected with serum, were normal again the next day.

If one centrifuges the mixture, the above mentioned liquid is not found to cause a swelling, but the precipitate is. So we have here an analogous conduct as with the corpuscles<sup>1)</sup>.

I have now tried whether specific albumen precipitations did not show the same conduct, and for this I chose the precipitates of horseserum with colloidal  $Fe(OH)_3$  and  $SiO_2$ . Both precipitates gave some swelling and at a morphological investigation polynucleous leucocytosis. This investigation must still be extended.

If one injects a prepared animal with specific serum, one gets the same phenomenon: swelling and leucocytosis. This phenomenon is wellknown. I did not yet succeed in proving here as well that the precipitins hold the serum in its place<sup>2)</sup>, although I do think it likely, considering what goes before. For the time being I do not see a chance of preparing a serum which possesses amboceptor against foreign albumen, but no precipitin.

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**Chemistry.** — “*The Temperature-coefficients of the free Surface-energy of Liquids at Temperatures from  $-80^\circ$  to  $1650^\circ$  C.*

**1. Methods and Apparatus.** By Prof. Dr. F. M. JAEGER. (Communicated by Prof. P. VAN ROMBURGH).

§ 1. The purpose of the experiments here described was to endeavour to ascertain the relation between the so-called “molecular surface-energy” of molten salts and the temperature, — a relation which has hitherto been studied only in liquids, which possess no electrolytical conductivity.

<sup>1)</sup> The experiments are somewhat analogous to those about the local effect of the anaphylatoxin (FRIEDBERGER), but I always used serum that was made inactive, contrary to the investigators, into the anaphylatoxin.

<sup>2)</sup> That is to say subconjunctively. For the cornea other laws probably prevail; there the serum remains in the same place for rather a long time without there being any precipitins (WESSELY, VON SZILY).